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July 22, 2004

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APPLICATION NUMBER: *60/483,279*
FILING DATE: *June 27, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/17979*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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Certifying Officer

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label N . EU115634909US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Yinhua	Zhang	Winchester, MA
Larry	McReynolds	Beverly, MA

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Specific Detection of Chitin in Chitin-Containing Organisms Using Chitin-Binding Domains

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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OR

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification *Number of Pages* 21 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 1 ☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 14-0740

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): 120.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Harriet M. Strimpel Date 6/26/03

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REGISTRATION NO. *(if appropriate)* 37008

Docket Number: NEB-231

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

Docket No.: NEB-231

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

THIS IS A PROVISIONAL APPLICATION

INVENTOR(S): Yinhua Zhang
Larry McReynolds

TITLE: SPECIFIC DETECTION OF CHITIN IN CHITIN-CONTAINING ORGANISMS USING CHITIN-BINDING DOMAINS

ATTORNEY: Harriet M. Strimpel, D.Phil.
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Specific detection of chitin in chitin-containing organisms using chitin-binding domains

THIS IS A PROVISIONAL APPLICATION

Chitin is one of the most abundant biopolymers in nature. Although chitin content varies significantly among different organisms, it is widely distributed among invertebrates including arthropods, nematodes, crustaceans, fungi and some protozoa. Detecting the presence of chitin is not a trivial task. Most currently available techniques use non-specific dyes such as fluorescent calcofluor and lectins, both of which bind also other polysaccharides. Consequently, a positive result obtained using these methods may not be specific for chitin. Therefore, a chitin-specific detection method is needed to reveal the true presence of chitin in chitin-containing organisms.

In searching for novel and specific ways to detect chitin, the proprietary chitin-binding domain (CBD), U.S. Patent No. 5,834,247, from New England Biolabs was tested for its suitability as a chitin specific detection reagent. This CBD has a high affinity for chitin and binds tightly and has been used as an affinity tag for protein purification. In the present invention, a CBD-based detection method was developed and shown to specifically detect chitin in the chitin-containing nematode *C. elegans*.

In the example described, 2 recombinant fusion proteins were tested containing GFP as the fluorescent reporter for CBD-based binding. GFP was fused to the N- terminus of CBD (GFP-CBD), or contained within a nuclear hormone receptor-CBD fusion protein (Nhr-GFP-CBD). In addition, both fusion proteins contain a spacer sequence (New England Biolabs, Inc., 2002/2003 Catalog, page 164, Product No. 6900S), intein, between GFP and CBD. GFP-

CBD and Nhr-GFP-CBD were each over-expressed in *E. coli*. Total protein lysates were prepared from bacterial cells expressing each fusion protein and directly used as the staining reagent.

Before staining, *C. elegans* whole worms representing different developmental stages were fixed and permeabilized using a modified immunocytochemistry method.

Worms were incubated with the above bacterial lysates for approximately 1 hour and then observed using microscopy. Specific staining was obtained in this short time frame and no significant increase was observed after 4 hours. As shown in Figure 1, strong staining is observed in the eggshells as predicted, and other structures such as the pharynx where chitin synthase expression has been reported. Λ

The successful use of CBD to detect chitin in *C. elegans* indicates that it can be used as a general tool to detect chitin in other organisms, such as parasites, fungi and protozoa. In principle, the CBD can be obtained from a native or recombinant source. It can be used as a full length protein, fusion or non-fusion, derivative or portion thereof, or synthesized.

In the present example, GFP was used to monitor specific binding of CBD to chitin. In principle, other labels can be used as known in the art. For example, other fluorescent dyes, enzymes conjugates, antibodies, or radioactivity. The CBD reagent can be used in *in situ* staining of intact cells, tissues or whole organisms, or used to detect chitin in cell lysates or manufactured chitin-containing products. These techniques will allow the detection of chitin in a qualitative or quantitative manner.

New England Biolabs, Inc.
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DECLARATION
AND POWER OF ATTORNEY
Original Application

Attorney Docket No. NEB-231

As a below named inventor, I hereby declare that:
My residence, post address and citizenship are as stated below next to my name
I believe that I am the original, first and sole inventor (in only one name is listed at 201 below) or an original, first and joint inventor (if plural names are listed at 201-203 below) of the subject matter which is claimed and which a patent is sought on the invention entitled:

Specific Detection of Chitin in Chitin-Containing Organisms Using Chitin-Binding Domains

which is described and claimed in:
[X] the attached specification or [ ] the specification in Application Serial No. _____ filed_____
(for declaration not accompanying application)
And was amended on_____
if applicable

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendments referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).
I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

FOREIGN APPLICATION(S) IF ANY, FILED WITHIN 12 MONTHS PRIOR TO THE FILING DATE OF THIS APPLICATION			
COUNTRY	APPLICATION	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119
			YES NO
			YES NO

ALL FOREIGN APPLICATION(S) IF ANY, FILED MORE THAN 12 MONTHS PRIOR TO THE FILING DATE OF THIS APPLICATION			
COUNTRY	APPLICATION	(day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (Patented, Pending, Abandoned)

DECLARATION
AND POWER OF ATTORNEY
PAGE 2 OF 3

POWER OF ATTORNEY:

As a named inventor, I hereby appoint the following attorney with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature of Inventor 201	Date 6/26/03
Signature of Inventor 202	Date 6/26/03
Signature of Inventor 203	Date
Signature of Inventor 204	Date
Signature of Inventor 205	Date
Signature of Inventor 206	Date
Signature of Inventor 207	Date
Signature of Inventor 208	Date
Signature of Inventor 209	Date

Figure 1. Specific chitin staining in *C. elegans* eggshell (Panel A) and pharynx (Panel B) using GFP-CBD. Arrows point to the stained chitin observed with fluorescent microscope (top) or corresponding cell structure observed with DIC microscopy (bottom). Early embryos do not stain because they lack chitin. Likewise, later embryos do not stain because of an additional protective layer.